REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED
November 13, 2002	Final Report	01 June 1998 - 31 May 2002

4. TITLE AND SUBTITLE

Graduate and Undergraduate Training in Marine Environmental Biotechnology

5a. CONTRACT NUMBER

5b. GRANT NUMBER
N000014-99-1-0312

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Kevin R. Sowers, PI

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Univ. of MD Biotechnology Institute, 701 E. Pratt St., Baltimore, MD 21202

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Office of Naval Research, 800 N. Quincy St., Arlington, VA 22217-5000

10. SPONSOR/MONITOR'S ACRONYM(S)
ONR

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AILABILITY STATEMENT

Distribution unlimited

13. SUPPLEMENTARY NOTES

20021202 008

14. ABSTRACT

The overall objective of this project was to enable students to participate and contribute to our ongoing research on the identification and characterization of microbes that catalyze polychlorinated biphenyl (PCB)-dechlorinating processes in the environment. The specific objective was to provide students at the graduate and undergraduate levels with skills that will enable them to conduct independent research in marine environmental molecular biology.

15. SUBJECT TERMS

PCBs, bioremediation, marine sediments, natural attenuation, microbial catalysts

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Kevin R. Sowers
Unclass.	Unclass.	Unclass.	UP	4	19b. TELEPHONE NUMBER (Include area code) 410.234.8878

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FINAL REPORT

GRANT #: N00014-99-1-0312

PRINCIPAL INVESTIGATORS: Kevin R. Sowers, Ph.D., University of Maryland Biotechnology Institute, Center of Marine Biotechnology, 701 E. Pratt St., Baltimore, MD 21202

GRANT TITLE: Graduate and Undergraduate Training in Marine Environmental Biotechnology

REPORT PERIOD: 01 March 1999 - 28 February 2002

OBJECTIVE: The overall objective of this research is to enable students to participate and contribute to our ongoing research on the identification and characterization of microbes that catalyze polychlorinated biphenyl (PCB)-dechlorinating processes in the environment. The specific objectives include training students with skills that will enable them to conduct independent research in environmental molecular biology.

APPROACH: Students were trained to perform cutting edge molecular technologies to study marine bioremediation including: (i) use of molecular DNA probes to further define microbial populations associated with specific transformation pathways; (ii) anaerobic isolation and characterization of PCB-dechlorinating microorganisms that can be used for mechanistic studies and bioaugmentation of contaminated sites; (iii) development of screening methods for rapid molecular monitoring of PCB dechlorinating potential and activity in the laboratory and *in situ*. Acquired skills included development of anaerobic microcosms from marine sediments, isolation of anaerobic marine microorganisms, cloning and sequencing of DNA, PCR amplification of DNA, development of gene probes, and computer sequence analyses of 16S rDNA sequences

ACCOMPLISHMENTS:

The microbial catalysts for many environmental processes such as the dechlorination of PCBs have been difficult to identify by traditional isolation techniques. To overcome this limitation, traditional enrichment techniques have been combined with molecular monitoring using amplified rDNA restriction analysis (ARDRA) of a clone library, denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (TRFLP) to identify the biocatalysts that reductively dechlorinate highly chlorinated and potentially toxic PCB congeners. Enrichment microcosms were initiated with three dioxin-like PCB congeners: 22'33'44'55' octachlorobiphenyl (PCB 194), 2,33'44'55' heptachlorobiphenyl (PCB 189) and 33'44' tetrachlorobiphenyl. PCBs 189 and 194 were tested for the potential of the dechlorination process to form toxic dioxin-like PCB 77 (toxification). PCB 77 was chosen to test for the potential of the dechlorination process to detoxify this dioxin-like congener.

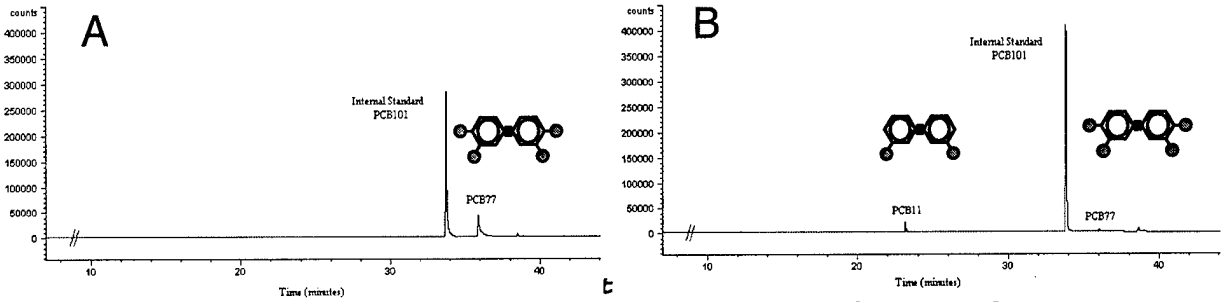

Figure 1. Reductive dechlorination of PCB 77 by Baltimore Harbor microcosms grown with acetate as and electron donor at 0 days (A) and 309 days (B).

After 309 day of incubation with fatty acids or acetate as electron donors PCB 77 was reductively dechlorinated to PCB 11, effectively transforming the toxic co-planar form of PCB to a less toxic non-coplanar form. The structure of the product has been confirmed by GC-MS (Figure 2). Result indicate that coplanar PCB77 is reduced by removal of two

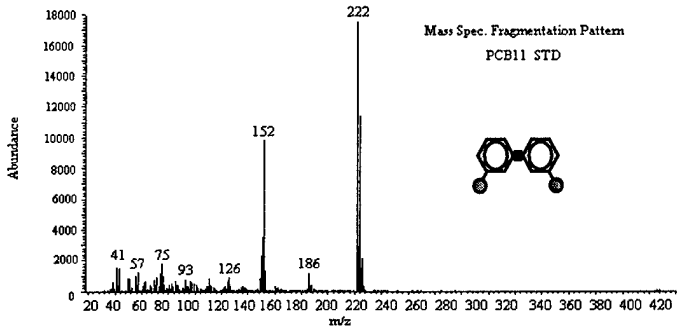

Figure 2. GC-MS analysis of PCB77 dechlorination product confirming that structure is PCB 11.

chlorines in the 55′ positions. Although intermediates were not detected, it is not possible to conclude that this is a one step process since the intermediates may not have accumulated at undetectable levels. This activity has been successfully transferred and analysis with our PCB dechlorinator-specific probes indicates that the catalyst is most closely related to other PCB dechlorinating species (Figure 3). Activities have not yet been detected for the highly chlorinated PCB congeners 189 and 194, but we are continuing to monitor the cultures.

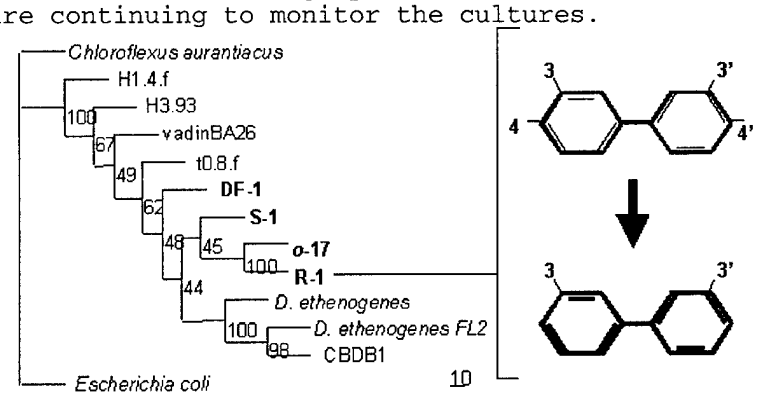

Figure 3. Phylogentic tree showing that the PCB77 dechlorianting catalyst (R-1) is most closely related to the other PCB dechlorinating species o-17, S-1, DF-1.

Bioaugmentation experiments by undergraduate Heather Allen have yielded promising results. In preliminary experiments sediments from Oslo Harbor that did not exhibit *ortho*-dechlorination were combined in a microcosm with an equal volume of Baltimore Harbor sediment in a mineral medium with only acetate as an electron donor. These microcosms exhibited *ortho*-dechlorination of 2356-CB, which is a signature reaction of indigenous BH sediment microbial communities. This established that the lack of *ortho*-dechlorination in Norwegian sediments was not due to inhibitory factors. The experiment were then repeated using highly enriched sediment-free cultures that contained the *ortho*-dechlorinating strain *o*-17, identified by the PIs in and ONR Harbor Processes funded project. Addition of this enriched culture to Oslo Harbor sediments catalyzed *ortho*-dechlorination of 2356-CB in the sediments. In order to determine whether the dechlorinating strain could be maintained within the indigenous microbial community, bioamended cultures depleted of 2356-CB were stored for a period of 6 months without replenishment of the PCB.

Thus far after 3 months, *ortho*-dechlorinating activity has not been restored. Molecular analyses are currently being conducted with molecular probes to determine whether the *ortho*-dechlorinating population has been diminished or requires an induction period in the presence of the PCB congener.

The students have been trained to perform cutting edge molecular technologies to study marine bioremediation including: (i) use of molecular DNA probes to further define microbial populations associated with specific transformation pathways; (ii) anaerobic isolation and characterization of PCB-dechlorinating microorganisms that can be used for mechanistic studies and bioaugmentation of contaminated sites; (iii) development of screening methods for rapid molecular monitoring of PCB dechlorinating potential and activity in the laboratory and *in situ*. Acquired skills have included development of anaerobic microcosms from marine sediments, GC/ECD and GC/MS analyses of PCBs and interpretation of data, cloning and sequencing of DNA, PCR amplification of DNA, development of gene probes, and computer sequence analyses of 16S rDNA sequences.

Mr. Hebert left the program on a medical leave, but has since been employed by Maryland Department of the Environment and most recently by the US postal service to conduct environmental research. Training from this program was directly responsible for Mr. Hebert's past and current positions. Another graduate student, Ms. Sonya Fagervold has continued the research outlined in this proposal. Ms Allen has graduated from the University of Maryland with honors and will continue to participate in the preparation of a manuscript describing her work on bioaugmentation. Her training in this program has lead to her current employment at Johns Hopkins University as a laboratory technician. She plans on applying to medical schools this year.

CONCLUSIONS: The students' research provides a basic understanding of the dehalogenating processes extant in coastal sediments using PCBs as a model system and the potential for these processes to 1) detoxify potentially harmful PCB congeners; and 2) stimulate dechlorinating by indigenous populations of microorganisms. The ultimate goals of the study were to understand the biocatalytic processes that transform PCBs in order to determine whether the indigenous microbial population has the potential to detoxify PCBs of concern and determine whether these processes are potentially amenable to biotechnological enhancement by bioaugmentation of PCB-impacted coastal harbors and sediments.

SIGNIFICANCE: Our initial studies on the dechlorination of PCB77 suggest that natural attenuation has the potential to detoxify sediments impacted by toxic coplanar PCBs. The results of this research will facilitate Navy management decisions concerning both remedial site prioritization and appropriate remedial strategies.

PATENT INFORMATION: No patents disclosures were filed.

AWARD INFORMATION: The PI (KRS) was promoted to the rank of associated professor during the course of this project.

PUBLICATIONS AND ABSTRACTS:

May,H.D.,Allen,H.,T.Briseid,O.Bergersen,G.Eidsa and K.R.Sowers. Effects of temperature on the reductive dechlorination of PCBs in microcosms from low temperature marine sediments. *In preparation.*

Watts,J.E.M.,R.Hebert, S.B.Schreier, H.D.May and K.R.Sowers. Distribution of PCB dechlorinating communities in PCB impacted marine sediments. *In preparation.*

Watts,J.E.M., May,H.D.,and Sowers,K.R. 2002. Development of a molecular screening technique for microorganisms that reductively dechlorinate PCBs